

**What is claimed is:**

1. A method of predicting pregnancy outcome in a subject by determining the amount of an early pregnancy associated molecular isoform of hCG in a sample comprising:

(a) contacting a sample with an antibody which specifically binds to the early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG;

(b) measuring the amount of complexes formed, thereby determining the amount of the early pregnancy associated molecular isoform of hCG in the sample; and

(c) comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (b) with either (i) the amount determined for temporally matched, normal pregnant subject(s) or (ii) the amount determined for non-pregnant subject(s), wherein the relative absence of the early pregnancy associated molecular isoform of hCG in the sample indicates a negative outcome of pregnancy for the subject.

2. A method of predicting the likelihood of a negative pregnancy outcome in a female subject comprising:

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- 5 (a) contacting a sample from the subject with a capture antibody which specifically binds to an early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG;
- 10 (b) contacting any complex formed in step (a) with a labelled detection antibody under conditions permitting binding to the complex the capture antibody and the hCG isoform;
- 15 (c) measuring the amount of labeled detection antibody bound to the complex so as to thereby determine the amount of the early pregnancy associated molecular isoform of hCG in the sample; and
- 20 (d) comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (b) with the amount determined for a normal pregnant subject, wherein the relative absence of the early pregnancy associated molecular isoform of hCG in the sample indicates a negative outcome of pregnancy for the subject.
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- 30 3. The method of claim 1, step (a) further comprising a second antibody which specifically binds to hCG without substantially cross-reacting with said antibody under conditions
- 35 permitting formation of a complex between the

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antibody and the early pregnancy associated molecular isoform of hCG.

4. The method of claim 3, wherein the capture antibody is B152.

5. The method of claim 4, wherein the detection antibody is B207.

10 6. A method of predicting the likelihood of a negative pregnancy outcome in a female subject comprising:

15 (a) contacting a sample from the subject with a capture antibody which specifically binds to an early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG;

20 (b) measuring the amount of complexes formed, thereby determining the amount of the early pregnancy associated molecular isoform of hCG in the sample; and

25 (c) comparing the amount measured in step (b) with the amount determined by contacting the same sample with a second capture antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG and a

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second detection antibody, wherein a high ratio of amounts determined for said first capture antibody relative to the second capture antibody indicates a positive outcome of pregnancy for the subject, a low ratio indicates a negative outcome of pregnancy for the subject.

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- 10 7. The method according to claim 5, wherein the second capture antibody is B109 and the second detection antibody is B108.
- 15 8. The method of claim 1, step (c) comprising comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (b) with either (i) the amount determined for temporally matched, normal pregnant subject(s) or (ii) the amount determined for non-pregnant subject(s), wherein amounts of the early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG in temporally matched pregnant samples indicates a positive outcome, amounts of early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG.
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- 30 9. The method of claim 1, wherein the sample is a urinary sample or a blood sample.
- 35 10. The method of claim 1, wherein the sample is an aggregate sample taken from at least one day.

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11. The method of claim 1, wherein the antibody is labeled with a detectable marker.
12. The method of claim 11, wherein the detectable marker is a radioactive isotope, enzyme, dye, magnetic bead, or biotin.
13. The method of claim 12, wherein the radioactive isotope is  $I^{125}$ .
14. A method of predicting pregnancy outcome in a subject by determining the amount of an early pregnancy associated molecular isoform of hCG in a sample comprising:
- (a) contacting a capturing antibody which specifically binds to the early pregnancy associated molecular isoform of hCG with a solid matrix under conditions permitting binding of the antibody with the solid matrix;
  - (b) contacting the bound matrix with the sample under conditions permitting binding of the antigen present in the sample with the capturing antibody;
  - (c) separating the bound matrix and the sample;
  - (d) contacting the separated bound matrix with a detecting antibody which specifically binds to hCG under conditions permitting binding of antibody and antigen in the sample;

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(e) measuring the amount of bound antibody on the bound matrix, thereby determining the amount of early pregnancy associated molecular isoform of hCG in the sample;

(f) comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (e) with either (i) the amount determined for temporally matched, normal pregnant subject(s) or (ii) the amount determined for non-pregnant subject(s), wherein amounts of the early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG in temporally matched pregnant samples indicates a positive outcome, amounts of early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG in the non-pregnant samples indicates a negative outcome of pregnancy for the subject.

15. The method of claim 14, further comprising:

(a) removing of the sample from the matrix; and

(b) washing the bound matrix with an appropriate buffer.

16. The method of claim 14, wherein the capturing antibody is E152.

17. The method of claim 14, wherein the detecting antibody is B207.

5 18. The method of claim 14, step (a) further comprising a second capturing antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG.

10 19. The method according to claim 18, wherein the second capturing antibody is B109.

15 20. The method of claim 14, step (d) further comprising a second detecting antibody which specifically binds to hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG.

20 21. The method of claim 14, step (f) comprising comparing the amount of the early pregnancy associated molecular isoform of hCG determined in step (e) for said antibody with the amount determined in step (b) for the second antibody, wherein a high ratio of amounts determined for said antibody relative to the second antibody indicates a positive outcome of pregnancy for the subject, a low ratio indicates a negative outcome of pregnancy for the subject.

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22. The method of claim 14, wherein the sample is a urinary sample or a blood sample.
- 5 23. The method of claim 14, wherein the sample is an aggregate sample taken from at least one day.
- 10 24. The method of claim 14, wherein the antibody is labeled with a detectable marker.
25. The method of claim 24, wherein the detectable marker is a radioactive isotope, enzyme, dye, magnetic bead, or biotin.
- 15 26. The method of claim 25, wherein the radioactive isotope is  $I^{125}$ .
- 20 27. A method for determining the amount of early pregnancy associated molecular isoforms of in a sample comprising:
- 25 (a) contacting the sample with an antibody which specifically binds to an early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG; and
- 30 (b) determining the amount of complexes formed thereby determining the amount of early pregnancy associated molecular isoform of hCG in the sample.
- 35 28. The method of claim 27, wherein the antibody specifically binds a region of the early



pregnancy associated molecular isoform of hCG comprising a carbohydrate moiety.

- 5      29.      The method of claim 27, wherein the antibody is produced by the hybridoma cell line accorded ATCC Accession No.HB-12467 .
- 10      30.      The method of claim 27, wherein the antibody is B152.
- 15      31.      A diagnostic kit for determining the amount of early pregnancy associated hCG is a sample comprising:
- 20              (a)      An antibody which specifically binds to an early pregnancy associated molecular isoform; and
- (b)      a solid matrix to which the antibody is bound; and
- 25              (c)      reagents permitting the formation of a complex between the antibody and a sample.
- 30      32.      The diagnostic kit of claim 31, wherein the antibody is B108 as detection antibody and B109 as capture antibody.
- 35      33.      The diagnostic kit of claim 31, wherein the antibody is B207 as detection antibody and B152 as capture antibody.
34.      The diagnostic kit of claim 31, 32 or 33 further comprising control sample(s) normal pregnant sample(s).

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35. The diagnostic kit of claim 31, 32, or 33 wherein the antibody is labeled with a detectable marker.
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36. The diagnostic kit of claim 35, wherein the detectable marker is a radioactive isotope, enzyme, magnetic bead, dye or biotin.
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37. The diagnostic kit of claim 36, wherein the radioactive isotope is I<sup>125</sup>.
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38. An antibody which specifically binds to an early pregnancy associated molecular isoform of human chorionic gonadotropin.
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39. The antibody of claim 38, wherein the antibody specifically binds to a region of the early pregnancy associated molecular isoform of human chorionic gonadotropin comprising both a carbohydrate moiety and protein part.
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40. The monoclonal antibody of claim 38 designated B152.
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41. A hybridoma cell accorded ATCC Accession No. HB-12467, producing the monoclonal antibody of claim 40.
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42. The early pregnancy associated isoform of hCG of claim 1.
43. The early pregnancy associated isoform of hCG recognized by the monoclonal antibody of claim 40.

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presence of non-trophoblast malignancy in the sample.

45. The method of claim 44, wherein the antibody is B152.

46. The method of claim 44, wherein the complex formed in step (a) is detected by a detection antibody.

47. The method of claim 46, wherein the detection antibody is B207.

48. The method of claim 44, wherein the second antibody is B109.

49. The method of claim 44, wherein the complex formed in step (b) is detected by a detection antibody.

50. The method of claim 49, wherein the detection antibody is B108.

51. The method of claim 44, wherein the non-trophoblast malignancy is ovarian malignancy or prostate malignancy or some other non-trophoblast malignancy.

52. The method of claim 44, wherein the sample is a urinary sample or a blood sample.

53. A method for detecting gestational trophoblast disease in a sample from a subject comprising:  
(a) contacting a sample with an antibody which specifically binds to the early pregnancy

associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG;

(b) contacting the sample with a second antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG;

(c) measuring the amount of complexes formed, thereby determining the amount of the early pregnancy associated molecular isoform of hCG in the sample due to binding with the first antibody, and late pregnancy associated molecular isoform of hCG in the sample due to binding with the second antibody;

(d) determining the ratio of early pregnancy associated molecular isoform of hCG to late pregnancy associated molecular isoform of hCG in the subject; and

(e) comparing the ratio of early pregnancy associated molecular isoform of hCG to late pregnancy associated molecular isoform of hCG in the sample determined in step (c) over time, wherein a continuing high ratio of early pregnancy associated molecular isoform of hCG to late pregnancy associated molecular isoform of hCG in the sample determined in step (c) indicates

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the presence of gestational trophoblast disease in the subject.

54. The method of claim 48, wherein the antibody is B152.
55. The method of claim 48, wherein the second antibody is B109.
56. The method of claim 48, wherein the gestational trophoblast disease is choriocarcinoma or hydatidiform mole.
57. The method of claim 48, wherein the sample is a urinary sample or a blood sample.
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58. An antibody which specifically binds to an early pregnancy associated molecular isoform of human chorionic gonadotropin.
59. The antibody of claim 58, wherein the antibody specifically binds to a region of the early pregnancy associated molecular isoform of human chorionic gonadotropin comprising a carbohydrate moiety.
60. The monoclonal antibody of claim 58 designated B152.
61. A hybridoma cell accorded ATCC Accession No. HB-12467, producing the monoclonal antibody of claim 60.

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62. The antibody of claim 58 labeled with a detectable marker.

5 63. The antibody of claim 58, wherein the detectable marker is a detection antibody.

64. The antibody of claim 58, wherein the antibody specifically binds to the nicked form of human gonadotropin.

10 65. The antibody of claim 58, wherein the antibody specifically binds an epitope dependent upon peptide bond cleavage in beta loop 2.

15 66. The antibody of claim 58, wherein the antibody binds the human gonadotropin at the beta COOH-terminal region.

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